

# First record of the suctorian ciliate *Ophryodendron abietinum* (Ciliophora, Phyllopharyngea), the epibiont of Hydrozoa in the White Sea

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| Submitted November 20, 2020 | Accepted December 22, 2020 |

## Summary

The species of the genus *Ophryodendron* are epibionts of various invertebrate animals. They are rarely found in nature, and very little research has been dedicated to them recently. *Ophryodendron abietinum*, an epibiont of a hydrozoan *Obelia longissima*, was discovered in the White Sea for the first time. This study provides a description of *O. abietinum* life cycle forms and host-epibiont interactions.

**Key words:** ciliates, epibionts, Hydrozoa, *Obelia longissima*

## Introduction

Suctorina is a subclass of ciliates (Ciliophora, Phyllopharyngea) with peculiar characteristics of the feeding process (suction) and cortex organization when only resettlement forms are ciliated (Bardele, 1970, 1972; Dovgal, 2002; Lynn, 2008). Some species of suctorians are epibionts of various organisms (Fernandez-Leborans et al., 1996, 2002; Dovgal, 2002; Dovgal et al., 2008; Sergeeva et al., 2014). Most epibiotic ciliates have not been studied in detail, and little is known about their biology and diversity. *Ophryodendron* spp. are an excellent example of the neglected suctorian epibionts whose

relationships with their basibionts remain undiscovered.

*Ophryodendron abietinum* was discovered on the hydrozoan colonies in 1859 (Claparède and Lachmann, 1859). Further studies on *O. abietinum* focused only on some aspects of general morphology (Hincks, 1873; Martin, 1909). The authors described the species and provided ideas about its possible life cycle consisting of two life forms: the probosciform and the vermiform. However, due to the rare registration of the species, the authors did not provide sufficiently detailed data. Other species of the genus *Ophryodendron* inhabit a wide range of marine invertebrates, including hydroids (Claparède and

Lachmann, 1859; Martin, 1909), crustaceans (Bâtisse and Dragesco, 1967; Bâtisse, 1969; Fernandez-Leborans et al., 1996, 2002), nemertines (Duboscq, 1925) and polychaetes (Wailes, 1925). During the 20<sup>th</sup> century, there were no general studies on the genus *Ophryodendron*, but some authors (Wailes, 1925; Jahn and Jahn, 1949) mentioned particular species. Only at the turn of the century, Fernandez-Leborans and co-authors published two articles (Fernandez-Leborans et al., 1996, 2002) where they provided the morphological description of *O. mysidacii* and its life cycle. The structure of the genus *Ophryodendron* and the whole family Ophryodendridae remains doubtful, and more data are required for a proper revision (Jankowski, 1981; Dovgal, 2002). Thus, the present study is the first detailed description of *O. abietinum* after its last discovery at the beginning of the 20<sup>th</sup> century.

## Material and methods

The study area is situated near the educational and research station “Belomorskaya” of Saint Petersburg State University at Sredniy Island, Chupa Bay, the White Sea, 66°17'21" N, 33°39'41" E (Fig. 1). The first site of sample collection was located along the shores of the strait between Bolshoy Gorely Island and the mainland, the second site - on the western coast of Vichennaya Luda Island. Both sites were chosen due to the presence of the abundant kelp forests of *Laminaria* sp. which serves as a substrate for *Obelia* colonies. The sampling was done during the end of July – beginning of August in 2018–2020.

For the first time, *O. abietinum* was discovered accidentally on fixed colonies of the hydrozoan *Obelia longissima* (Pallas, 1766), typical for the White Sea, in 2018 near Bolshoy Gorely Island (Fig. 1, site 1). The colonies were collected and preserved in 4% paraformaldehyde (PFA) in 0.1 M phosphate-buffered saline (PBS). After fixation, specimens were transferred to PBS solution with 0.1% NaN<sub>3</sub> for conservation. During subsequent light and fluorescent (stained with DAPI) microscopic investigation of *Ob. longissima*, numerous individuals of *O. abietinum* species were found. However, while in the summer of 2019 we repeatedly found *O. abietinum* at the same collection point, in 2020 we only discovered *O. abietinum* at the western coast of Vichennaya Luda Island (Fig. 1, site 2).

The samples of the kelp thalli with rhizoids were collected by boat hooking from the depth of

5–10 meters. The kelp pieces with *Ob. longissima* colonies were cut off for further observation in laboratory conditions. Additionally, colonies of the closely related littoral hydrozoan species, *Gonothyraea loveni* (Allman, 1859), were collected from the brown algae *Ascophyllum nodosum* and *Fucus vesiculosus* at the same sites. The observation of the collected hydrozoan colonies was conducted with Leica EZ4HD stereo microscope (Leica Microsystems GmbH, Germany).

The photographs of the live specimens were taken with Leica DM500 microscope (Leica Microsystems GmbH, Germany) with Samsung Galaxy A3, CMOS 13.0 mp phone camera. The photographs of the nematocysts in cells cytoplasm were taken with Leica TCS SP5 Laser Scanning Confocal Microscope (Leica Microsystems GmbH, Germany), the photographs of fixed cells were taken with Nikon Eclipse Ni equipped with a DS-Fi3 camera (Nikon, Japan). All the morphometric measurements were made using the material fixed in 2018 with FiJI ImageJ program (Babraham Institute, UK). Not less than 10 cells from each morphological form were analyzed.

The cells fixed in 4% PFA were used for the scanning electron microscopy. The specimens were subsequently dehydrated in series of ethanol solutions and acetone, critically point-dried in Hitachi critical point dryer HCP-2, placed on stubs coated with platinum using Giko IB-5 Ion coater, and viewed under FEI Quanta 250 scanning electron microscope.

## Results and discussion

**Systematics** (according to Lynn, 2008).

Class Phyllopharyngea de Puytorac et al., 1974  
 Subclass Suctorina Claparède et Lachmann, 1859  
 Order Exogenida Collin, 1912  
 Family Ophryodendridae Stein, 1972  
 Genus *Ophryodendron* Claparède et Lachmann, 1859  
*Ophryodendron abietinum* Claparède et Lachmann, 1859

## Description

We found two different stages of *O. abietinum* life cycle (Fig. 2), represented by two distinct forms of cells: proboscidiform and vermiform. We identified both forms as *O. abietinum* based on general morphological features and hydrozoan host

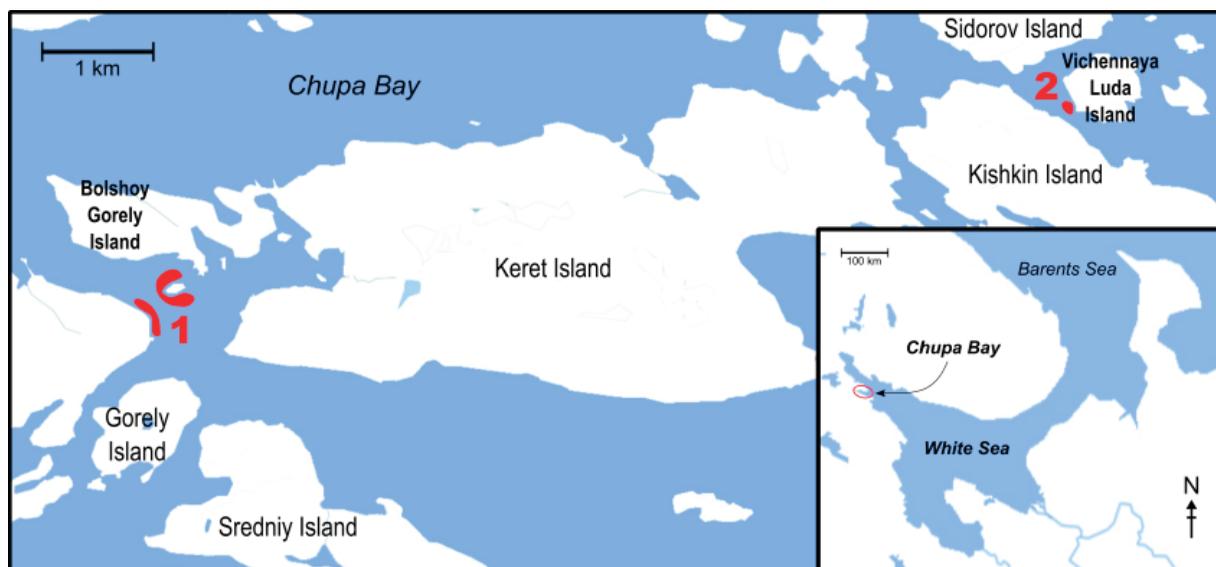


Fig. 1. The map of the sampling points near Sredniy Island, Chupa Bay, the White Sea.

specificity (Claparéde and Lachmann, 1859; Martin, 1909). Furthermore, we discovered the budding cells in fixed material (Fig 2, D) and observed the early stages of budding in living specimens. This finding conveys that both stages belong to the same species.

According to the modern views, proboscidiform and vermiform cells are the stages of the typical suctorian life cycle (Jankowski, 2007; Lynn, 2008). It includes trophont, the attached feeding stage, which forms a short-lived settling swarmer by different types of budding (Dovgal, 2002). In the Ophryodendridae family, trophont is represented by a proboscidiform cell, while swarmer is represented by a vermiform cell. Unlike other suctorian swarmers, which are much smaller than trophonts, the swarmers of *Ophryodendron* are of the same size as trophonts and do not possess cilia (Jankowski, 2007). They are formed on trophont by vermigemmy, which is considered a variation of exogemmic budding (Dovgal, 2002, 2013).

Trophonts are pear-shaped,  $65 \pm 15 \mu\text{m}$  in length and  $35 \pm 8 \mu\text{m}$  in width. They possess a feeding apparatus formed by a single actinophore with 23–30 tentacles (Fig. 2, B, E). Normally the actinophore stretches out and can reach the edges of the polyp's hydrotheca. In disturbed or fixed specimens, the actinophore contracts forming multiple folds, whereas the tentacles always remain straightened. In a contracted state, the length of the actinophore is  $25 \pm 7 \mu\text{m}$ . The trophont attaches to the host's perisarc with a basal disk and never forms a stalk.

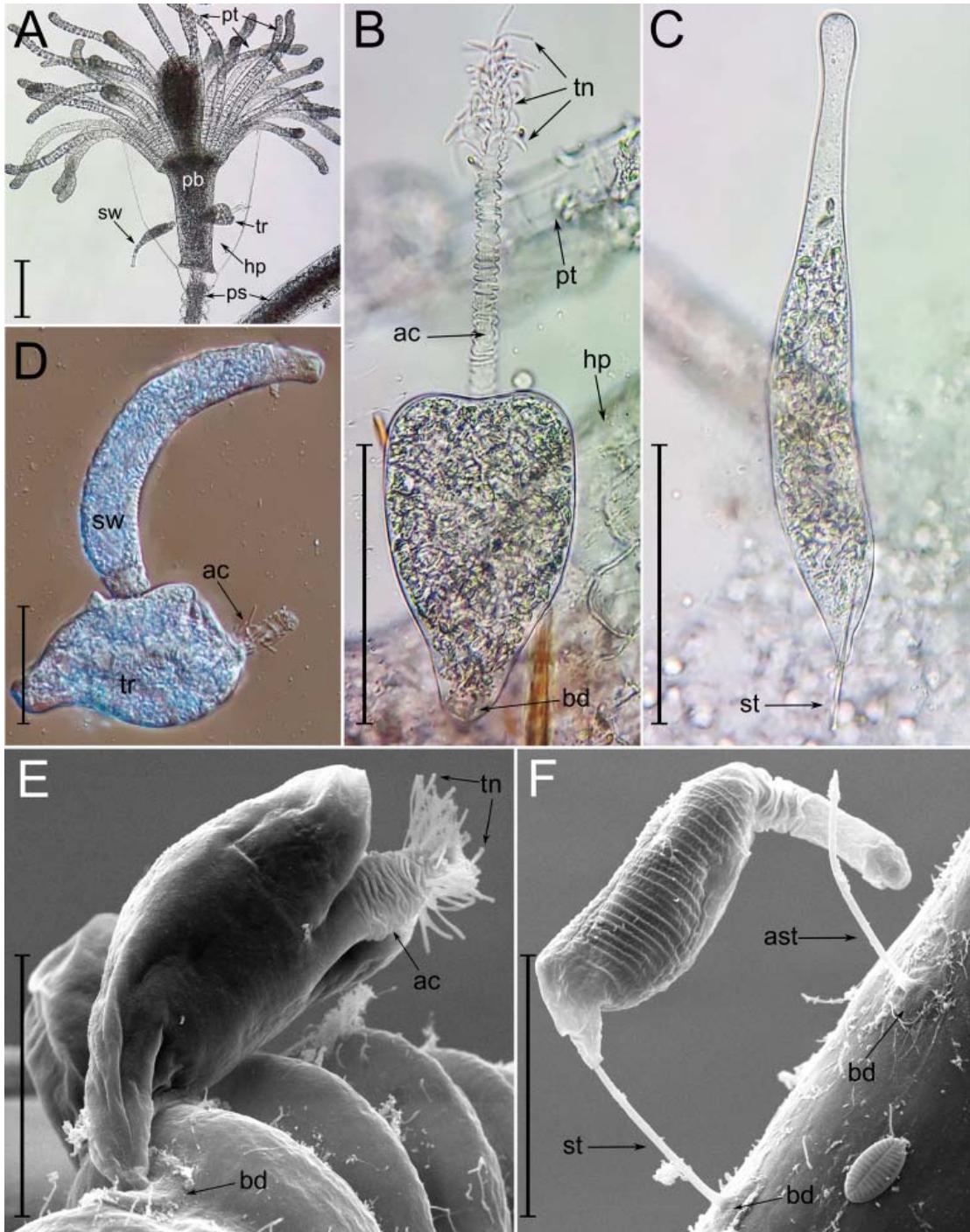
The basal disk is inconspicuous in live specimens, but it is well distinguishable in fixed cells (Fig. 2, E).

Worm-like swarmers reach  $107 \pm 15 \mu\text{m}$  in length and  $26 \pm 8 \mu\text{m}$  in width (Fig. 2, C, F). They do not have a feeding apparatus or tentacles. Swarmers always form stalks with a basal disc. Cells can easily detach from these stalks as evidenced by the presence of abandoned stalks on the surface of the perisarc (Fig. 2, F).

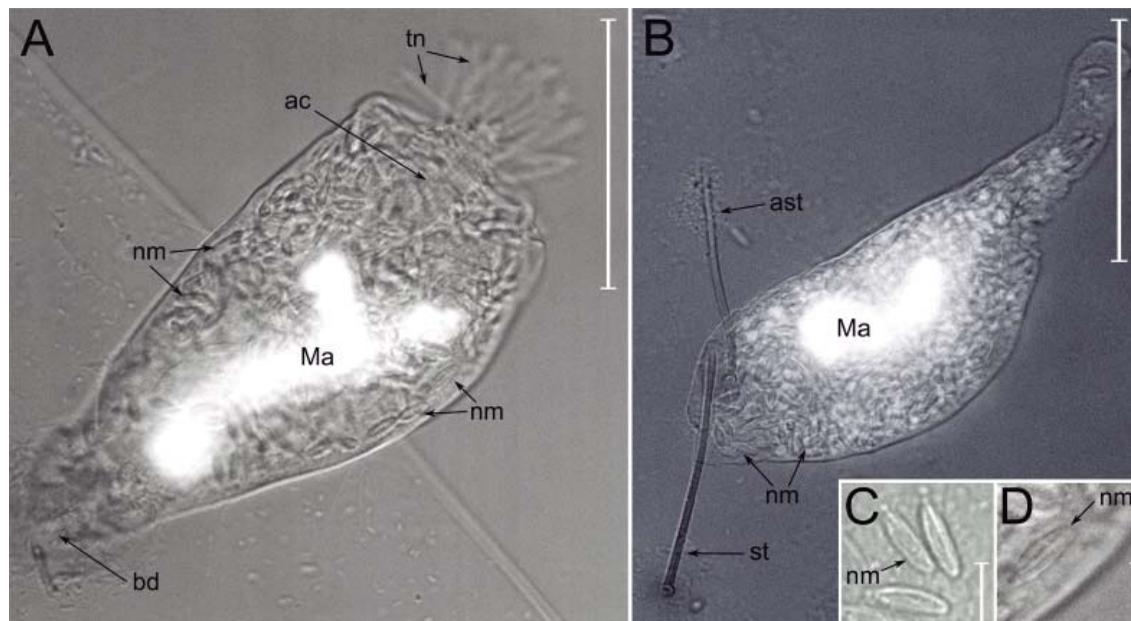
### Host-epibiont interaction

Both stages of *O. abietinum* life cycle inhabit the same parts of the *Ob. longissima* colony. Most of the specimens are typically found in the middle part of the feeding polyp's (gastrozooids) hydrotheca. Less often the cells are located on the thin branches' perisarc near the hydrotheca. We never observed *O. abietinum* on the older parts of the colony which are covered by a thick perisarc layer and on the growing polyps, gonozooids (polyps producing jellyfish), or empty hydrotheca.

The nature of the relationship between *O. abietinum* and its host is unclear. Numerous suctorian species, including members of the genus *Ophryodendron*, are epibionts of a wide range of animals, for example, crustaceans or mites (Fernandez-Leborans and Tato-Porto, 2000, 2002; Chatterjee et al., 2018). Several species inhabit cnidarian colonies or even jellyfish bell surface (Flood et al., 2015). They use the colony's perisarc as a suitable substrate and compete



**Fig. 2.** Different stages of the *O. abietinum* life cycle. A–C – Live specimens, D – cells fixed in 4% PFA, E–F – SEM photographs. A – Trophont and swarmer at the hydrotheca of the *Ob. longissima* feeding zooid, B – trophont with elongated actinophore, C – swarmer. D – vermigemmic budding process, E – trophont with highly contracted actinophore, F – swarmer and abandoned stalks at the perisarc of *Ob. longissima*. *Abbreviations:* ac – actinophore, ast – abandoned stalk, bd – basal disk, hp – hydrotheca of the polyp, pb – polyp's body, ps – stalks of the polyp's colony, pt – polyp's tentacles, st – stalk, sw – swarmer, tn – tentacles of the actinophore, tr – trophont. Scale bars: A – 100  $\mu$ m, B–F – 50  $\mu$ m.



**Fig. 3.** Nematocyst in cells of different stages of *O. abietinum*, specimens fixed in 4% PFA and stained with DAPI. A – Trophont, B – swarmer, C – nematocyst in the tentacle of *Ob. longissima*, D – nematocysts in the cell of *O. abietinum*. Abbreviations: Ma – macronucleus, nm – nematocyst capsule, for explanation of other symbols see Fig. 2. Scale bars: A–B – 50  $\mu$ m, C–D – 10  $\mu$ m.

for food resources with gastrozooids (Tazioli and Di Camillo, 2013).

*O. abietinum* and related species were initially considered to be parasites of hydrozoans (Claparède and Lachmann, 1859; Hincks, 1873). This conclusion was based on the presence of multiple nematocysts (stinging capsules of cnidarians), which are particularly well distinguishable in fixed cells (Fig. 3). The unusual feeding process – trophonts absorbing the content from epidermal cells of the cnidarian host tentacles – was observed by Martin (1909, 1914). However, trophonts catch prey like other suctorians living on the cnidarian colonies (Tazioli and Di Camillo, 2013). Martin (1909) also mentioned the cases of *O. abietinum* capturing small ciliates. The feeding process of swarmers has not been described, neither in *O. abietinum* nor in other species of Ophryodendridae. Similar to swarmers of other suctorians, *O. abietinum* swarmers probably do not feed. Nevertheless, we found nematocysts in all of the observed cells at both stages (Fig. 3). Possibly, swarmers inherit nematocysts from trophonts during budding.

The specificity of the interaction between *O. abietinum* and its host is also uncertain. Historically, *O. abietinum* was found on various hydrozoan spe-

cies: *Obelia* sp. (Claparède and Lachmann, 1859; Roof, 1922), *Clytia* sp. (Martin, 1909), *Kirchenspaueria pinnata* – originally *Plumularia pinnata*, *Halecium halecinum* (Hincks, 1873). Detailed descriptions and drawings of *O. abietinum* are absent in most of these works, which date back over a century. Moreover, these hydrozoans live in highly contrasting environmental conditions and have different colony organization, particularly gastrozooids' morphology and arrangement (Antsulevich, 2015).

Over three years, we found *O. abietinum* exclusively on the colonies of *Obelia longissima* and never observed it on the colonies of *Gonothyrea loveni*, a closely related species. *Ob. longissima* and *G. loveni* are members of the subfamily Obelinae and were previously placed in the genus *Obelia* due to their high morphological similarity (Bouillon et al., 2004). Both species are abundant in the White Sea but inhabit different ecological niches: *Ob. longissima* is typically found at the depth of more than five meters in *Laminaria* sp. kelp forests, whereas *G. loveni* grows on the brown algae *Ascophyllum nodosum* and *Fucus vesiculosus* in the littoral zone. We suppose that the host preference of *O. abietinum* is determined not only by the host morphology but also

by environmental conditions. In this case, epibiotic ciliates may use hydrozoan species as hosts only if they occupy the appropriate ecological niche.

To conclude, our data show that the two stages of the complex life cycle of the suctorian ciliate *O. abietinum* are regularly found on the colonies of hydrozoan *Ob. longissima* in the White Sea. Could this interaction be an example of obligate parasitism accompanied by strong epibiont-host specificity between a suctorian and the hydrozoan, or is it a solely ecological framing? This question is still an enigma, and the actual relationships between *O. abietinum* and its hydrozoan host remain to be unveiled in the future studies.

## Acknowledgements

The research was carried out at the “Centre for Culture Collection of Microorganisms” of Research Park of St. Petersburg State University as well as the Core Facilities Centre “Taxon” of the Zoological Institute Russian Academy of Sciences, St Petersburg, Russia. We thank Dr. A.A. Dobrovolsky for helping with the first identification of *O. abietinum* and inspiration for this study. This study was supported with the budgetary grant of the Zoological Institute RAS No AAAA-A19-119031390116-9.

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